

# Influence of motor complete spinal cord injury on visceral and subcutaneous adipose tissue measured by multi-axial magnetic resonance imaging

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**Objective:** Abdominal obesity conveys substantial health risks, in association with high levels of visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT) and an increased proportion of VAT to SAT. The purposes were to determine the influence of spinal cord injury (SCI) on the associations between single axial cross-sectional area (CSA) slices and the average CSA or volumes of VAT and SAT across multi-axial slices of magnetic resonance imaging (MRI); and the relationships relative to the whole body composition and anthropometrics.

**Methods:** Thirteen healthy male participants with traumatic motor complete SCI underwent fast spin-echo MRI to measure VAT and SAT across multi-axial slices, followed by dual-energy X-ray absorptiometry to measure whole body fat-free mass (FFM) and fat mass (FM). Waist circumference (WC) was also measured in the seated position.

**Results:** The trunk CSAs of VAT and SAT were  $99 \pm 51$  and  $164 \pm 69$  cm<sup>2</sup>, respectively, and the ratio of VAT to SAT was  $0.68 \pm 0.33$ . The CSAs of VAT and SAT at a single slice strongly predicted the average CSA and modestly predicted the volumes across multi-axial slices. VAT and SAT represented  $5.7 \pm 1.8\%$  and  $9.7 \pm 3.2\%$  of the total body FM, respectively. Percent body FFM was negatively related to VAT and SAT volumes, but not to a single axial CSA.

**Conclusion:** A single slice CSA can modestly predict the volume of multi-axial slices in individuals with SCI, yet it is not related to any of the body composition variables. Increased percent FFM is associated with a reduction in VAT and SAT volumes measured across multi-axial slices. The ratio of VAT to SAT is greater than 0.4, suggesting that individuals with SCI are at high risk of developing metabolic sequelae.

**Keywords:** Spinal cord injuries, Body composition, MRI, DXA, Subcutaneous and visceral adipose tissue, Obesity

## Introduction

Spinal cord injury (SCI) is a devastating medical condition that affects approximately 10 000–11 000 individuals on an annual basis.<sup>1</sup> It is widely accepted that there is a decline in fat-free mass (FFM) associated with skeletal muscle atrophy and increase in fat mass (FM) after SCI.<sup>2–5</sup> Individuals with SCI have total body FM that exceeds 27%,<sup>4–7</sup> and when compared to age and body mass index (BMI)-matched able-bodied (AB) controls, they have 1.13-fold more fat per unit of BMI.<sup>5</sup> Altered body composition after SCI is associated with impaired glucose tolerance,<sup>8,9</sup> insulin resistance,<sup>10–12</sup>

dyslipidemia,<sup>10,13</sup> osteoporosis,<sup>14</sup> metabolic syndrome,<sup>15</sup> and consequently, cardiovascular disease.<sup>4–16</sup> These pathological adaptations contribute to the increased socioeconomic burden, decreased quality of life and shortened life expectancy after SCI.<sup>17</sup> Therefore, studying the factors that may lead to these metabolic sequelae is of paramount importance to this population.

Central or abdominal obesity is commonly associated with deterioration in metabolic, cardiovascular and other health-related variables.<sup>18–24</sup> The reported contribution of visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) to different metabolic disorders varies widely<sup>25</sup> and is still under investigation after SCI. Much of this variability can be attributed to differences in the methodological accuracy of quantifying VAT and SAT. The distribution of VAT and SAT

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was previously predicted using waist circumference (WC), the waist-to-hip ratio,<sup>21,22,24,26,27</sup> or using more advanced imaging techniques such as ultrasound to measure adipose tissue depth, dual-energy X-ray absorptiometry (DXA) to quantify trunk adiposity,<sup>11,28,29</sup> or more recently, highly precise quantitative techniques such as computerized tomography (CT) and magnetic resonance imaging (MRI).<sup>30–41</sup> MRI is now identified as the gold-standard technique that could easily quantify the volumes of VAT and SAT.<sup>32,35,37,40</sup> Ross and colleagues have demonstrated the use of MRI to capture multi-axial slices to quantify the volumetric distribution of VAT and SAT. Their data support the robustness of a very brief scan MRI protocol to measure VAT and SAT.<sup>32–34</sup>

The cross-sectional areas (CSAs) of VAT and SAT were recently measured in individuals with SCI using single axial CT scans at the level of L4–L5 or umbilicus.<sup>30,31</sup> Despite similarities in BMI and WC between the two studies,<sup>30,31</sup> there are wide discrepancies in the values reported in VAT CSA. Edward *et al.* reported that the range of CSAs of VAT and SAT may vary up to 89 and 87%, respectively, among individuals with SCI.<sup>30</sup> The use of a single slice compared to multi-axial slices remains an issue of controversy in the process of quantifying VAT and SAT.<sup>37–40</sup> Lee *et al.* suggested that a single slice can accurately predict SAT or VAT mass.<sup>41</sup> Abate *et al.* showed that measuring SAT at L4–L5 space had the poorest correlation of the SAT volume compared to L5–S1 space.<sup>37</sup> This may suggest that the CSA of a single slice may not accurately reflect the magnitude of VAT and SAT after SCI.

We are unaware of any study that has quantified VAT and SAT volumes in individuals with SCI using multi-axial slices. Considering the array of adaptations in body composition after SCI,<sup>4,5,22,42</sup> it is unclear if the CSA of a single slice can predict the distribution of VAT and SAT across multi-axial slices. Additionally, we have quantified the distribution of VAT and SAT relative to the whole body FM in individuals with SCI. We hypothesized that a single slice may predict the CSA of multi-axial slices; however, it may not accurately reflect the volumes of VAT and SAT. Additionally, the relative distribution of VAT and SAT would be lower than what has been previously reported in AB controls due to the greater whole body FM in individuals with SCI.

## Materials and methods

### Participants

Thirteen healthy men with chronic traumatic motor complete SCI (mean  $\pm$  SD age  $35 \pm 8$  years, body weight  $74 \pm 13$  kg, height  $182 \pm 7$  cm, and BMI  $23 \pm$

$4 \text{ kg/m}^2$ ) participated in the study. Only men were chosen to ensure homogeneity of our sample and to reduce possible effects of sex on body composition.<sup>26,43</sup> Additionally, compared to women, men accumulate greater quantities of VAT.<sup>43</sup> The participants were at least 1 year post-injury with levels of injury ranging from C5 to T11 and American Spinal Injury Association (ASIA) classification A or B (*motor complete SCI*). We have chosen to study motor complete SCI because the magnitude of body composition adaptations may vary between complete and incomplete SCI.<sup>2,3,5</sup> Participants were recruited from the University of Michigan SCI model system ( $n = 5$ ; C6–T11) and from Indiana University Hospitals ( $n = 8$ ; C5–T11). Participants signed an informed consent statement that was approved by the local ethics committee at the University of Michigan and Indiana University and they were compensated for participation.

Participants were included if they were (1) men aged 18–45 years of age, with the maximum age chosen to avoid any confounding effects of the aging process on body composition; (2) a minimum of 1 year post-injury, due to adaptations in body composition stabilizing by this time<sup>5</sup>; and (3) the level of injury was C5–T11; individuals with injury above C5 have limited hand functions and are dependent on others to prepare their meals, which may indirectly influence their body composition, body weight, VAT, and SAT.<sup>32,34</sup> Lower motor neuron injury is typically found in those with injury below T11 and leads to flaccid paralysis of the involved skeletal muscles that ultimately affects body composition.<sup>44</sup>

Participants were excluded from the study if they had any of the following characteristics: had cardiovascular disease, hypertension, diabetes, or high lipid profile, were smokers or alcohol abusers, or had pressure ulcers greater than Grade II. Individuals with BMI greater than  $30 \text{ kg/m}^2$  were excluded because lower BMI cut-off points have been recommended to identify the risk of obesity in people with SCI.<sup>5,6</sup> Persons with MRI-incompatible materials such as rods, screws, valves, and stents that were implanted for different medical purposes were also excluded.

### Anthropometric variables

All study participants received a physical examination including a resting electrocardiogram and basic vital signs. Body weight was measured while wearing light clothes in a supine position using a hospital bed scale calibrated to 0.1 kg. Height was measured from the same position to the nearest 0.1 cm. BMI was calculated as weight in kg divided by height in  $\text{m}^2$  ( $\text{kg/m}^2$ ). WC ( $n = 9$ ) was measured using inelastic tape measure at

the level of the narrowest part of the torso.<sup>22</sup> The measurements were taken in doublets from a seated position in their wheelchairs at the end of expiration without compressing the skin as was previously done.<sup>22</sup> All measurements were obtained by the same investigator.

#### *Dual-energy X-ray absorptiometry*

DXA was used to study the whole body FFM and FM (kg). Body composition was measured using whole body scans with a Lunar Prodigy Advance scanner ( $n = 5$ ; Lunar DPX, DXA Scanner; Lunar Inc., Madison, WI, USA) and a Hologic QDR-2000 scanner ( $n = 8$ ). Selection of two different densitometers was based on the availability of the DXA scanners at the two institutions. To account for any variability, a whole body standard phantom (Body Phantom S/N 1067, Hologic, Inc., Waltham, MA, USA) was scanned 26 times to correct for the source of error from using two different densitometers. The results showed that the between-machine difference was 0.06% for FM and 7% for FFM. Whole body %FM and FFM were calculated after excluding bone tissue and the index of FM to FFM was used to determine relative distribution of FM. The coefficient of variability of two repeated scans on the same participant was less than 3%.

#### *Tissue measurement by MRI*

Images were obtained with a 1.5 or 3 T whole body scanner (General Electric Signa scanner, Milwaukee, WI, USA). T1-weighted imaging was performed using a fast spin-echo sequence with the following parameters: axial in-phase/out-phase with a repetition time of 140 ms and echo time of 4.2 and 1.8 ms for the in-phase and the out-phase, respectively; a 46 cm field of view, matrix size of  $256 \times 256$  or  $320 \times 320$ , number of excitations = 1 and acquisition time of 4–5 minutes. Transverse slices (0.8 or 1 cm thickness) were acquired every 0.4 or 1 cm gap from the xiphoid process to the femoral heads. Images were acquired in a series of two stacks with L4–L5 used as a separating point. After the acquisition of a localizer sequence, the inter-vertebral space between the fourth and fifth lumbar vertebrae was identified by locating the umbilicus.<sup>32–34</sup> To ensure a short breath holding duration, two sets of nine slices were captured. The first set extended superiorly from L4–L5 to the xiphoid process and the second set distally from L4–L5 to the femoral heads. During scanning, participants were asked to take a deep breath in and hold their breath for 10–15 seconds. The breath holding technique was applied to reduce the respiratory motion artifact normally

associated with acquisition of MRI in the abdominal region. The use of a fast spin echo and dividing the trunk region into two stacks ensured a short breath holding time for the participants, notably more tolerable for those with higher SCI.

#### *Procedures for acquiring MRI*

After arriving for study, participants were transported to the scanner for a non-contrast abdominal MRI. Participants were then asked to fill out an MRI safety checklist to ensure no contraindications. The MRI table was moved outside the magnet room and two persons (one MRI technician and one researcher) assisted in the participant's transfer from the wheelchair to the table. After lying supine, their knees and feet were strapped to ensure neutral position inside the magnet and to avoid incidental movement due to spasms, which may cause image artifact. The movable table was then docked to the magnet with the participant's head slide in first and arms were placed across the chest due to range of motion limitations preventing overhead placements. The technician instructed participants to maintain their position and to avoid movement during scanning. All participants were provided ear-plugs to protect against magnet noise and a blanket to maintain normal body temperature and to avoid triggering muscle spasms inside the magnet.

#### *Calculation of VAT and SAT area and volume*

Images were downloaded to a disk and analyzed on specifically designed software (Win Vessel 2, Ronald Meyer, PhD, Department of Physiology, MSU, East Lansing, MI, USA). The images were automatically segmented into fat (high-intensity), muscle (mid-intensity), and background/bone (low-intensity) regions.<sup>3,9,44,45</sup> The CSAs were computed automatically by summing the tissues' pixels and multiplying by pixel surface area (Fig. 1). Pixel surface area is multiplied by (Field of view/Matrix size)<sup>2</sup>. The volume (cm<sup>3</sup>) was calculated by multiplying the CSA by the slice thickness and interslice space (1.2 or 2 cm). Depending on torso length of the participants, 10–14 transverse images extended distally from the liver to the femoral heads were analyzed. Selection of the images was based on visual distinction of VAT and SAT regions within a single slice. To adjust for differences in torso length among our participants, consecutive slices were averaged in taller individuals and truncal VAT and SAT volumes were calculated by summing the volumes of 10 consecutive slices. The VAT and SAT masses (g) were calculated by multiplying the corresponding volumes (cm<sup>3</sup>) by fat density (0.92 g/cm<sup>2</sup>).<sup>46</sup>



**Figure 1** Representative axial MRI slice of L4-L5 space of individuals with C7 motor complete SCI (A) before and (B) after segmenting the image and tracing the region of interest. VAT CSA is the white region segmented within the abdominal wall and SAT CSA is the white region outside the abdominal wall. BMI: 23 kg/m<sup>2</sup>; WC: 84 cm; VAT: 66 cm<sup>2</sup>; SAT: 223 cm<sup>2</sup>.

### Reliability

Inter-observer reliability values were used to determine variations in the average CSA of VAT and SAT across multi-axial slices.<sup>41</sup> This was assessed by comparing the measurement outcomes of two trained examiners after each had analyzed multi-axial slices of two randomly selected participants. Using the coefficient of variation (CV), the inter-observer error was 13% for VAT and 1.5% for SAT. It should be noted that CV of a single examiner was no more than 3% for VAT and 0.5% for SAT. The reported values were within the acceptable range previously noted in a similar study.<sup>41</sup>

### Statistical analysis

Statistical procedures were performed using SPSS version 15.0 (SPSS, Chicago, IL, USA). Linear regression analysis was used to determine the relationship between single and multi-axial slice measures of VAT and SAT CSAs. Multiple regression analyses were used to determine the variables that could predict VAT or SAT volumes. One-way analysis of variance (ANOVA) was used to identify the statistical differences among three stacks of multi-axial slices from L1-L3, L3-L5, and L5-S1. The first and last stacks had three slices of axial images and L3-L5 had four slices. The arrangement of axial slices into stacks was

performed to determine the influence of anatomical sites on the magnitude of VAT and SAT accumulation. Pearson correlation was used to quantify the relationships between VAT or SAT and body composition variables.

### Results

#### Physical characteristics of the participants

Six participants had cervical (C5-C7, 46%) SCI and seven had thoracic (T4-T11, 54%) SCI. Out of the 13 individuals, 10 were ASIA A and the other 3 were ASIA B. The age and BMI ranged from 22 to 45 years and from 16 to 29 kg/m<sup>2</sup>, respectively. Time since injury was 12 ± 8 years (2-19 years post-injury).

#### Relationship between VAT and SAT and physical characteristics

Age was positively related to VAT volume ( $r = 0.60$ ,  $P = 0.03$ ) with a trend towards VAT CSA (0.54,  $P = 0.054$ ), but not with SAT or VAT:SAT ratio. Time since injury was positively related to VAT:SAT ratio ( $r = 0.64$ ,  $P = 0.019$ ). Table 1 presents the relationships among weight, BMI, and WC and the CSAs and volumes of VAT and SAT. It should be noted that the data of WC on four of the participants were inadvertently lost. WC was related to weight ( $r = 0.81$ ,  $P = 0.009$ ),

**Table 1** Relationship between CSAs (cm<sup>2</sup>) and volumes (cm<sup>3</sup>) of multi-axial slices and physical characteristics

	Cross-sectional areas			Volumes		
	SAT (cm <sup>2</sup> )	VAT (cm <sup>2</sup> )	SAT + VAT (cm <sup>2</sup> )	SAT (cm <sup>3</sup> )	VAT (cm <sup>3</sup> )	SAT + VAT (cm <sup>3</sup> )
Weight (kg)	0.63 (0.22)	0.66 (0.016)	0.80 (0.001)	0.60 (0.03)	0.68 (0.01)	0.75 (0.003)
BMI (kg/m <sup>2</sup> )	0.61 (0.027)	NS	0.71 (0.007)	0.77 (0.002)	0.70 (0.007)	0.89 (0.0001)
WC (cm)	0.67 (0.051)	NS	NS	0.74 (0.02)	NS	0.68 (0.046)

BMI: body mass index; WC: waist circumference; NS: non-significant values. Values shown are Pearson  $r$  ( $P$  values).

BMI ( $r = 0.85$ ,  $P = 0.004$ ), SAT volume, and sum of SAT and VAT volumes, but not to VAT volume (Table 1). Surprisingly, WC was related the SAT CSA at L3 ( $r = 0.75$ ,  $P = 0.02$ ) and L3–L4 ( $r = 0.71$ ,  $P = 0.03$ ), but not to L4–L5 and L5–S1.

#### Multi-axial distribution of VAT and SAT

Table 2 presents the distribution of VAT, SAT, and VAT:SAT ratio across multi-axial slices. The average CSAs of VAT and SAT were  $99 \pm 51$  and  $165 \pm 68$  cm<sup>2</sup>, respectively. The average volumes of VAT and SAT were  $1.4 \pm 0.6$  and  $2.4 \pm 1.1$ , respectively. VAT and SAT masses were  $1.3 \pm 0.6$  and  $2.2 \pm 0.9$  kg, respectively. VAT and SAT masses constitute  $5.7 \pm 1.8\%$  (2.5–8.5%) and  $9.7 \pm 3.2\%$  (4.5–14%) of the total body FM, respectively. One-way ANOVA did not identify any difference in VAT or SAT CSA after being separated into stacks of axial slices. The CSA of VAT ranged from 81 to 115 cm<sup>2</sup> between the inter-vertebral space of L1–L2 to L3 vertebral body and reached another peak towards S1, suggesting that accumulation of VAT is site dependent. SAT distribution showed a peak at L3–L4 space and L4 vertebral body with a decline towards the sacral region. The average ratio of VAT to SAT was  $0.68 \pm 0.30$  with the highest ratio at the sacral region above the femoral heads (Table 2). The high ratios of VAT to SAT suggest that these individuals are at high risk of developing metabolic abnormalities.

#### Prediction of multi-axial slices using a single slice

The CSA of a single slice at L3–L4, L4–L5, and L5–S1 modestly predicts VAT volume across multi-axial slices (Fig. 2A). However, the CSAs of L3–L4 ( $r^2 = 0.95$ ,  $P < 0.0001$ ) and L4–L5 ( $r^2 = 0.92$ ,  $P < 0.001$ ) strongly predict the average CSA of SAT across multi-axial slices, with a modest prediction at L5–S1 ( $r^2 = 0.57$ ,  $P < 0.003$ ). The average CSA of the stack of slices between L3–L5 and L5–S1 appeared to be more predictive of VAT volume compared to L1–L3 (Fig. 2B). The CSAs of a single slice at L3–L4, L4–L5, and L5–S1 also appear predictive of SAT volume across multi-axial slices (Fig. 3A). The same slices L3–L4 ( $r^2 = 0.93$ ,  $P < 0.0001$ ), L4–L5 ( $r^2 = 0.89$ ,  $P < 0.0001$ ), and L5–S1

( $r^2 = 0.92$ ,  $P < 0.003$ ) strongly predict the average CSA of SAT across multi-axial slices. The relationships of the stack of SAT slices at three different regions of the trunk and the SAT volume are presented (Fig. 3B). The L3–L5 region appeared to have the highest magnitude of prediction to the SAT volume ( $r^2 = 0.70$ ,  $P < 0.0001$ ) compared to L1–L3 ( $r^2 = 0.42$ ,  $P = 0.01$ ) and L5–S1 ( $r^2 = 0.55$ ,  $P = 0.004$ ) regions. Whole truncal SAT and VAT were not significantly related at the level of a single slice or across multi-axial slices.

#### Relationships between VAT or SAT and body composition variables

Percent body FFM and FM were  $67 \pm 5\%$  and  $31 \pm 6\%$ , respectively, and the index of FM to FFM was  $0.47 \pm 0.13$ . Percent body FFM was negatively related to VAT ( $r = -0.66$ ,  $P = 0.014$ ) and SAT ( $r = -0.72$ ,  $P = 0.005$ ; Fig. 4A) volumes and SAT CSA ( $r = -0.62$ ,  $P = 0.02$ ). Whole body FM was positively related to VAT and SAT volumes ( $r = 0.76$ ,  $P = 0.003$ ; Fig. 4B), and VAT ( $r = 0.61$ ,  $P = 0.02$ ) and SAT ( $r = 0.69$ ,  $P = 0.009$ ) CSA across multi-axial slices. Additionally, the ratio of FM to FFM was positively related to VAT ( $r = 0.66$ ,  $P = 0.014$ ) and to SAT ( $r = 0.72$ ,  $P = 0.006$ ) volumes (Fig. 5), but not to their CSAs. A single slice of SAT CSA at L3–L4, L4–L5, and L5–S1 was not related to any of the body composition variables. A single slice of VAT CSA at L3–L4 was related to FM ( $r = 0.65$ ,  $P = 0.016$ ). Finally, multiple regression analyses were used to identify the most significant predictors that could quantify VAT and SAT volumes (Table 3).

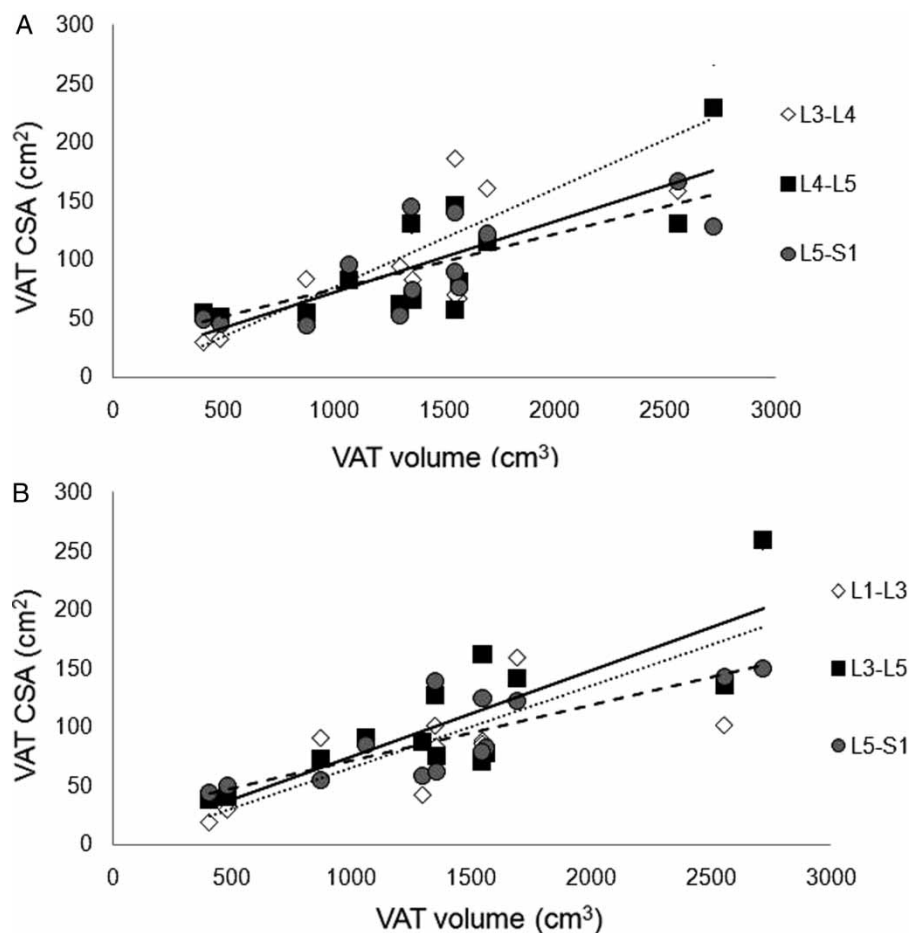
#### Discussion

The major findings of the study were that (1) the CSA of a single slice at L3–L4, L4–L5, or L5–S1 can strongly predict the average CSA across multi-axial slices and modestly predict the volumes of VAT and SAT; (2) VAT and SAT volumes exhibited expected relationships with %FFM and FM, but these were not evident with the CSA of a single slice; (3) maintaining a relatively high body FFM is associated with less accumulation of VAT and SAT volumes after SCI; (4) increased

**Table 2** Distribution of the VAT, SAT CSAs, and VAT/SAT ratio across multi-axial slices

	L1–L2	L2	L2–L3	L3	L3–L4	L4	L4–L5	L5	L5–S1	S1
VAT (cm <sup>2</sup> )	81.5 ± 59.5	95 ± 62	105 ± 66	115 ± 65	112 ± 68	99 ± 58	97 ± 52	86 ± 51	94.5 ± 42	97 ± 33
SAT (cm <sup>2</sup> )	129.5 ± 81	148 ± 80	166 ± 79	181 ± 79	188 ± 84	189 ± 76	190 ± 71	169 ± 66	147 ± 62	131 ± 60
VAT/SAT ratio	0.70 ± 0.45	0.73 ± 0.45	0.71 ± 0.44	0.7 ± 0.4	0.66 ± 0.38	0.58 ± 0.33	0.57 ± 0.31	0.56 ± 0.33	0.71 ± 0.3	0.89 ± 0.5

Values represent means ± SD for the CSA of axial slices. Letter L and S refer to lumbar and sacral vertebrae. The corresponding numbers refer to each vertebral body or inter-vertebral space.



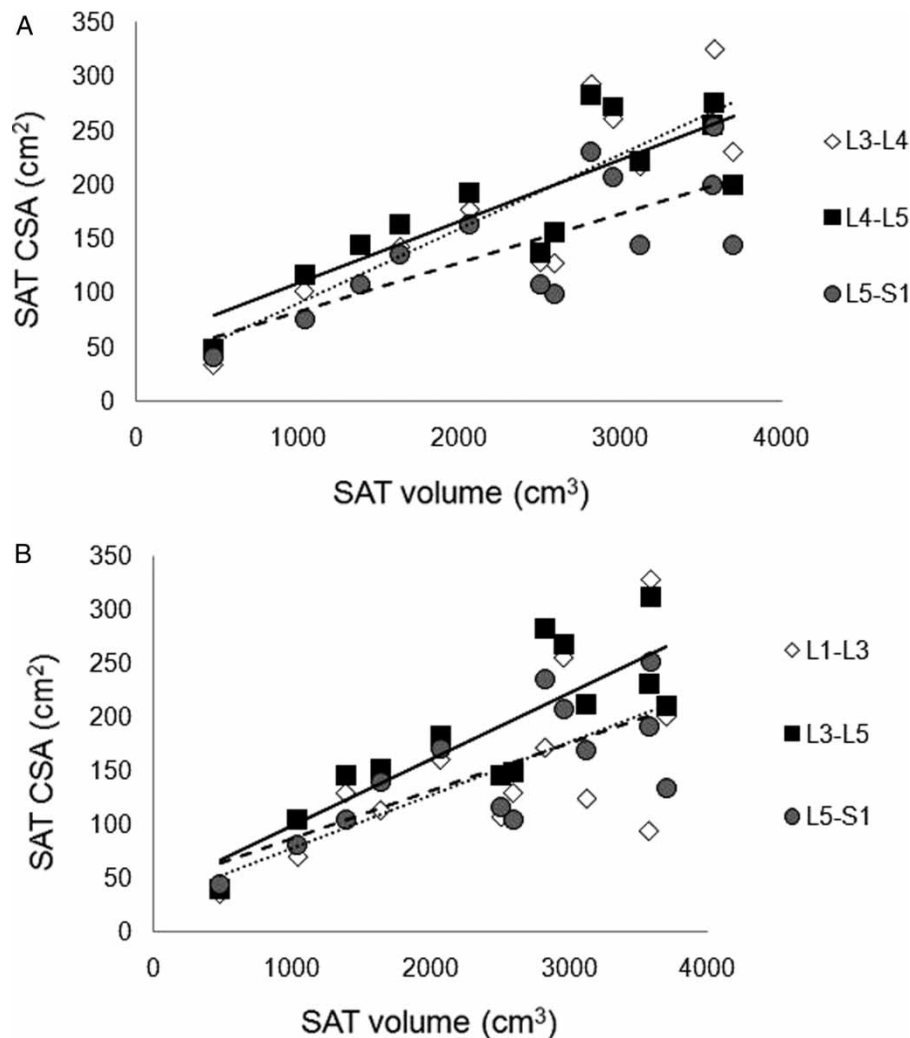
**Figure 2** Prediction of VAT volume using (A) single slice CSA at L3–L4 (dotted line,  $r^2 = 0.67$ ,  $P < 0.0001$ ), L4–L5 (solid line,  $r^2 = 0.62$ ,  $P < 0.001$ ), and L5–S1 (dashed line,  $r^2 = 0.56$ ,  $P < 0.003$ ) spaces and (B) stack of slices CSA at L1–L3 (dotted line,  $r^2 = 0.59$ ,  $P < 0.002$ ), L3–L5 (solid line,  $r^2 = 0.67$ ,  $P < 0.001$ ), and L5–S1 (dashed line,  $r^2 = 0.67$ ,  $P < 0.001$ ).

weight, BMI, and whole body FM are indicative of increased SAT and VAT volumes in people with SCI, but WC should be used with caution; and (5) the unfavorable ratio of VAT to SAT suggests that these individuals are at high risk of developing metabolic abnormalities.

Altered body composition after SCI increases the susceptibility to several deleterious metabolic disorders.<sup>8–16</sup> The importance of accurately evaluating body composition after SCI has been a subject of numerous investigations.<sup>5–7,11,42</sup> Compared to healthy or several other clinical populations, the contribution of VAT and SAT to altered metabolic and cardiovascular profile after SCI is poorly defined. Therefore, accurately quantifying the magnitude of distribution of VAT and SAT may help us to better understand the contribution to various metabolic pathogenesis after SCI. VAT and SAT CSAs have recently been quantified using a single slice CT scan at the level of L4–L5.<sup>30,31</sup> Edward *et al.*<sup>30</sup> suggested that VAT CSA is 120 cm<sup>2</sup> with a range of 27–257 cm<sup>2</sup>. Maruyama *et al.*<sup>31</sup> showed that VAT CSA is 191 cm<sup>2</sup>

and it can reach 300 cm<sup>2</sup>. These discrepancies in VAT CSA could possibly be explained by the fact that a single slice CSA cannot always capture the actual magnitude of VAT CSA. There is still growing skepticism that a single slice CSA can accurately predict the CSA of multi-axial slices or VAT and SAT volumes.<sup>37,38,40</sup>

We have described volumetric quantification of VAT, SAT, and the ratios for the first time in individuals with SCI using multi-axial MRI slices. Previously, multi-axial slices were used to quantify the extent of atrophy and hypertrophy after complete and incomplete SCI.<sup>2,3,9,45,47</sup> Moreover, MRI allows non-invasive quantification of adipose tissue such as intramuscular,<sup>3,9,45</sup> subfacial, and SAT.<sup>9,45,48</sup> In agreement with previous reports, a single CSA was able to accurately predict the average CSA across multi-axial slices; however, it modestly predicts the actual volume. Using a single slice CSA at L3–L4 improved the magnitude of relationship compared to other sites. It appeared that stacked slices between L3 and L5 can best predict VAT or SAT volumes. This may explain previous

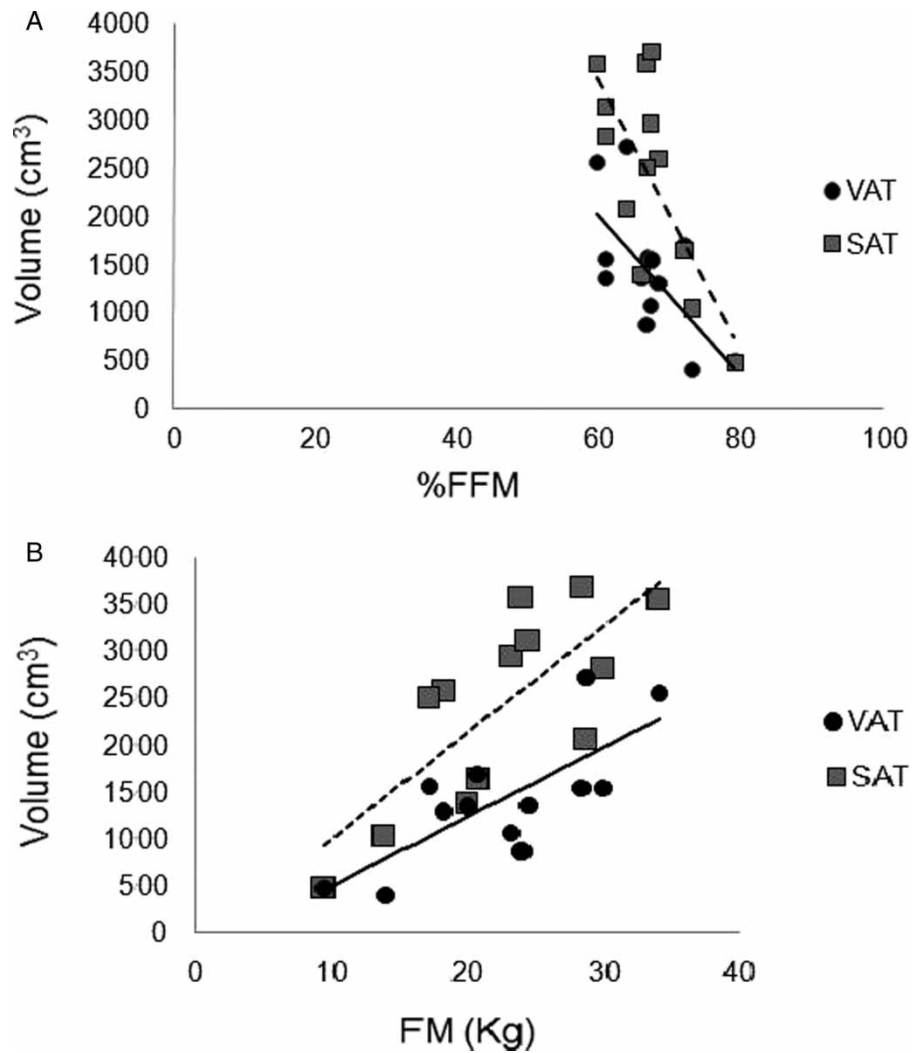


**Figure 3** Prediction of SAT volume using (A) single slice CSA at L3–L4 (dotted line,  $r^2 = 0.72$ ,  $P < 0.0001$ ), L4–L5 (solid line,  $r^2 = 0.68$ ,  $P < 0.0001$ ), and L5–S1 (dashed line,  $r^2 = 0.56$ ,  $P < 0.003$ ) spaces and (B) stack of slices CSA at L1–L3 (dotted line,  $r^2 = 0.42$ ,  $P < 0.01$ ), L3–L5 (solid line,  $r^2 = 0.71$ ,  $P < 0.0001$ ), and L5–S1 (dashed line,  $r^2 = 0.54$ ,  $P < 0.004$ ).

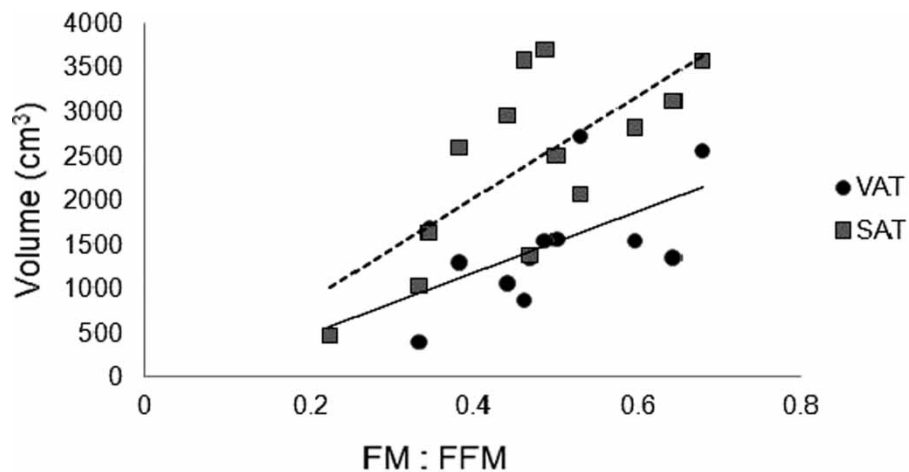
inconsistent relationships between the metabolic profile and VAT or SAT after using a single axial slice at L4–L5.<sup>30,31</sup> The use of MRI to capture multi-axial slice could have been hampered by the following challenges. First, individuals with rods in their back for spinal column fusions could experience complications or produce low-quality MR images. This was overcome by including individuals who have no rods in their back or have short rods that do not interfere with the region of interest causing imaging artifact. The second challenge was the difficulty in executing breath holding techniques, especially in higher level SCI. Selection of a fast spin-echo sequence and dividing the region of interest into stacks of slices allowed for a short breath holding duration.

VAT and SAT masses were diluted by an increase in whole body FM after SCI and especially FM in the

lower extremities, which constitute approximately 6% and 10% of total body FM, respectively. In AB controls, abdominal SAT represents 20–23% and VAT represents 10–13% of total body FM in lean and obese individuals.<sup>18</sup> Significant correlations were found between whole body FM and the two compartments of VAT and SAT. These relationships provide supporting evidence that an FM equal to or greater than 20 kg or 30% of the whole body FM may result in VAT CSA greater than 100 cm<sup>2</sup>. A previous epidemiological study that used a single slice showed that in men with VAT greater than 71 cm<sup>2</sup>, there is a risk of coronary artery calcification that may predict coronary artery diseases.<sup>27</sup> A novel finding of the current study is that high %FFM is associated with a reduction in VAT and SAT volumes. Similarly, intramuscular fat has been shown to be reduced after evoking skeletal muscle hypertrophy



**Figure 4** Relationship between SAT and VAT volumes and whole body composition (A) %FFM and (B) absolute body FM. Significant inverse relationship was noted between %FFM and VAT (solid line,  $r = -0.66$ ,  $P < 0.01$ ) or SAT (dashed line,  $r = -0.73$ ,  $P < 0.005$ ) volume. Whole body FM was positively related to either VAT (solid line,  $r = 0.7$ ,  $P < 0.003$ ) or SAT (dashed line,  $r = 0.76$ ,  $P < 0.003$ ) volume.



**Figure 5** Relationship between whole body ratio of FM:FFM and VAT (solid line,  $r = 0.66$ ,  $P < 0.01$ ) or SAT (dashed line,  $r = 0.72$ ,  $P < 0.006$ ) volume.



**Table 3** Multiple regression equations used to identify predictors of VAT and SAT volumes

Equations		
VAT volume (cm <sup>3</sup> ) =	23 (age) + 21 (weight) + 32.5 (%FM) – 1930 9 (age) – 1.9 (weight) + 37 (%FM) + 6.5 (L <sub>3</sub> –L <sub>4</sub> CSA)* – 648	R <sup>2</sup> = 0.63, P = 0.025 R <sup>2</sup> = 0.81, P = 0.006
SAT volume (cm <sup>3</sup> ) =	25 (weight) + 127 (%FM) – 42 (age) – 1929 4 (weight) + 83 (%FM) – 21 (age) – 7 (L <sub>3</sub> –L <sub>5</sub> CSA)** – 1027.	R <sup>2</sup> = 0.71, P = 0.009 R <sup>2</sup> = 0.81, P = 0.005

\*It should be noted that the use of L<sub>3</sub>, L<sub>3</sub>–L<sub>4</sub>, L<sub>4</sub>, and L<sub>4</sub>–L<sub>5</sub> CSAs is considered a significant predictor for measuring VAT volume.

\*\*The use of a single slice CSA did not improve the prediction of SAT volume compared to the average CSA of the four slices between L<sub>3</sub> and L<sub>5</sub>.

to the paralyzed muscles using neuromuscular electrical stimulation.<sup>45</sup> Moreover, a strong relationship was recently observed between whole body FFM and basal metabolic rate in individuals with SCI.<sup>7</sup> These findings may highlight the importance of developing counter-measures that could help in increasing skeletal muscle size or maintaining FFM in this population.<sup>45,47</sup> Previous work showed that resistance training results in reduction in VAT or SAT volumes, with preferential loss in VAT.<sup>32–34</sup> Relatively high FFM could be associated with increased testosterone or growth hormone that influences the distribution of VAT or SAT.<sup>49</sup>

Although BMI has been shown to underestimate the %FM in individuals with SCI,<sup>5,6,42</sup> the current study showed that increase in body weight or BMI is associated with increase in VAT and SAT. Moreover, WC was previously used to investigate central adiposity and the associated metabolic abnormalities after SCI.<sup>11,22</sup> In recreationally active athletes with SCI, WC was found to be related to insulin area under the curve and lipid profile.<sup>11</sup> In that study, the authors found a negative relationship between trunk FM and insulin sensitivity. Although WC was recently found to be highly correlated with VAT after SCI,<sup>30</sup> similar to previous reports<sup>34,35</sup> we could not replicate these findings. WC was only related with SAT volume and the sum of SAT and VAT, but not VAT. WC measurement was carried out in the seated position, possibly redistributing tissue into the waist region because of an absence of normal abdominal wall musculature. Limiting measurements to only the seated position without including a measurement in the supine position may have played a role in the lack of correlation with VAT. Losing WC data on four participants may also have affected the magnitude of the relationships.

The ratio of VAT to SAT suggests that these individuals are at high risk of developing metabolic disorders. Previously, a VAT-to-SAT ratio greater than 0.4 was suggested as cut-off points for those at risk of developing metabolic disorders.<sup>23</sup> MRI was recently used to quantify VAT and SAT distribution in those with type 2 diabetes. Those with worse insulin resistance have a greater

VAT and lower SAT phenotype profile.<sup>48</sup> We have observed a reduction in SAT CSA and an increase in the VAT CSA towards the sacral region similar to what has recently been reported in those with type 2 diabetes. It is unclear if the relative distribution of VAT to SAT is impacted by the level of SCI. One month post-injury, VAT increased in the T3 SCI rats but not in the T10 rats.<sup>36</sup> Future trials should investigate the effects of the level of injury on the distribution of VAT and SAT, especially in those with T9 or lower SCI. Moreover, the presumptive validity and reliability of DXA at L1–L4 should be determined compared to multi-axial MRI slices of VAT and SAT.

### Limitations

Data collections at two different institutions resulted in the use of two different sources of densitometers. Five participants were scanned on a Lunar and the rest were scanned on a Hologic scanner. In a previous study that measured body composition in individuals with SCI in relation to controls, it was suggested that Hologic overestimated FM compared to a Lunar scanner and that the primary sites of difference were the arms and legs.<sup>5</sup> We scanned a whole body phantom to adjust for any source of error between the two scanners; the results showed that the outcomes between both scanners are too trivial to influence any of the studied relationships. However, it is worth mentioning that differences between machines for FM and FFM in humans are likely to be greater than those determined by phantom measurements alone. Another limitation is the use of two different magnets with differing magnetic field strength (1.5 T vs. 3 T), which may have introduced a source of error. Spatial quantification of VAT and SAT depends primarily on the relaxation properties of lipid during MRI. de Bazelaire *et al.* showed insignificant difference in the relaxation time of SAT between 1.5 and 3T.<sup>50</sup> The lack of an AB control group may have limited the ability to compare the results of MRI abdominal VAT and/or SAT for the general population reported by other investigators. Nevertheless, there are two studies that have made

direct comparisons in VAT and SAT between individuals with SCI and AB controls. The results showed that individuals with SCI had 40% greater VAT, but insignificant difference in SAT CSA.<sup>30,31</sup> According to our research findings, the MRI technique can be applied to measure regional body composition adaptations in individuals with SCI. A metallic implant that extends down to L1–L2 may possibly cause visual artifacts that impede the clarity of the images to be analyzed. However, several research groups have successfully applied the techniques to measure skeletal muscle size,<sup>2,3</sup> intramuscular fat,<sup>9</sup> visceral and subcutaneous fat. In the current study, the technique appeared to be successful as long as there were no interfering implanted rods or screw and plates within the region of interest.

### Conclusions

The CSA of a single slice at the level of L3–L4, L4–L5, and L5–S1 can modestly predict SAT and VAT volumes across multi-axial slices. The findings suggest that a single slice CSA does not correlate with body composition similar to the volume of VAT or SAT. The significance of this finding is highlighted in the inverse relationship that was found between %FFM and both VAT and SAT volumes. VAT and SAT represent only 6 and 10%, respectively, of the whole body FM. This may suggest that both compartments are diluted by the higher percentage of FM in individuals with SCI. However, the ratio of VAT to SAT distribution suggests that individuals with SCI are at high risk of developing metabolic disorders.

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